DNA Markers for identifying *Bemisia tabaci* B and Q biotypes originated from various locations in Israel

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INTRODUCTION

In a survey of various *B. tabaci* (Hemiptera: Aleyrodidae) populations from Israel during 1999-2000, the Q biotype was recognized in addition to the biotype B (3). The key requirement for study of genetic diversity of *B. tabaci* is to devise a simple and cost-effective marker technology to distinguish between these biotypes. For this aim, we have developed, applied and compared various DNA markers. For developing a SCAR (sequence characterized amplified regions) technique, we used single RAPD (random amplified polymorphic DNA) fragments of B and Q biotypes. The CAPS (cleaved amplified polymorphic sequences) were investigated on the basis of sodium channel gene and COI (cytochrome oxidase I) gene sequences of both biotypes. We are applying and comparing these DNA markers in order to study population dynamics of *B. tabaci* biotypes.

AIM

Developing a rapid high throughput molecular diagnostic for B and Q biotypes of *B. tabaci*, which can be used for biological and ecological studies with this pest.

MATERIALS and METHODS

Origin of the whiteflies

The whiteflies used in this study, taken from established colonies previously described (3) and also collected in 2003-2006 from several regions in Israel, were preserved in 80% ethanol.

DNA extraction and RAPD-PCR reactions

DNA of individual adults was extracted according to a previously developed protocol (1) with some modifications. Reactions were made as follows: denaturation at 94°C for 1 min, annealing at 37°C for 1 min, and extension at 72°C for 2 min. The primer OPA-06 (5'-GGTCCCTGAC-3') amplified several PCR products that appeared unique to either *B. tabaci* biotype Q (931 bp) or B (1327 bp) (Fig. 1). DNA was analyzed by electrophoresis on horizontal 1-2% agarose slab gels with 1×TBE buffer for 2h at 70V and visualized with ethidium bromide.

Standards

As references to Q and B biotypes, we used *B. tabaci* individuals: from Israel laboratory populations, Pyri-R and Pyri-S (1), respectively; UK (JIC) and Italy (CNR).

RESULTS and DISCUSSION

Development of SCAR and CAPS markers

The primer OPA-06 amplified two biotype B specific bands and four biotype Q specific bands (Fig. 1). Two of the fragments were chosen for further analysis: the 1327 bp fragment specific to biotype B, and the 931 bp product specific to biotype Q (Fig. 1). These PCR products were cloned and sequenced. Based on the sequences, we designed a set of locus specific PCR primers (SCARs).

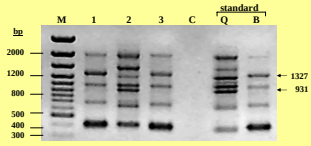

Fig. 1. RAPD-PCR analysis of individual of *B. tabaci* DNA samples with operon primer OPA-06.

M, 100 bp DNA Ladder Plus;
lanes 1 & 3, samples obtained from Sde-Eliahu and Beit Dagan;
lane 2, a sample obtained from Hof Carmel;
C, a control without DNA;
Q, a sample from reference Q biotype from population Pyri-R;
B, a sample from reference B biotype from population Pyri-S.

Arrows mark the position of Q and B specific bands.

In the B-type, the PCR reaction with primers: D1-B1 and R2-B1new resulted in a 1064 bp fragment (Fig. 2A; lanes 1 & 2). Thus, this set of primers could be useful for distinguishing B biotype from Q. However, in the Q-biotype, PCR amplifications using primers: D1-Q6, R1-Q6 and R2-Q6 resulted in a single monomorphic PCR products in both biotypes (D1-Q6 with R1-Q6 in 477 bp fragments and D1-Q6 with R2-Q6 in 683 bp fragments). Although the PCR product could not be used for direct genotyping of *B. tabaci* samples, digestion of the PCR products with endonuclease *Msp*I resulted in an appropriate polymorphism: cut-off from product PCR in case of the biotypes B and Q fragments 61 bp (lanes 1 & 3) and 39 bp (lanes 2 & 4), respectively (Fig. 2B).

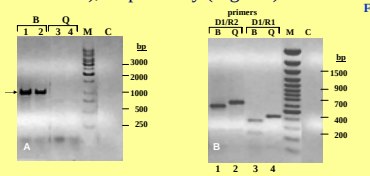

Fig. 2. A. SCAR analysis based on primers D1-B1 and R2-B1new.

Lanes 1 & 2, samples from population Pyri-S (B biotype);
lanes 3 & 4, samples of populations from Pyri-R (Q biotype);
M, 1 kb DNA Ladder; C, control without DNA.

Arrow marks the position of B specific band.

B. CAPS analysis using different primer combinations, following digestion with *Msp*I.

D1-Q6 & R2-Q6 primers: lanes 1 & 2, samples from population Pyri-S & Pyri-R, respectively;
D1-Q6 & R1-Q6 primers: lanes 3 & 4, samples from population Pyri-S & Pyri-R, respectively;
M, 100 bp DNA Ladder Plus; C, control without DNA.

CAPS on a basis of sodium channel gene sequence

Morin *et al.* (5) identified two mutations in the IIS4-5 linker of the *para*-type sodium channel of the *B. tabaci* strains resistant to a pyrethroid plus organophosphate mixture. Here, we used published oligonucleotide primer sequences kdr-1 and kdr-A970 (5) for amplification, cloning and analyzing sodium channel gene sequences from the two biotypes in Israel. Digestion of the 850 bp PCR product with *Asu*I resulted in an appropriate inter-sequence polymorphism: the 380 bp fragment which appeared with 470 bp (lanes 1, 2 and references B biotype (Fig. 3)).

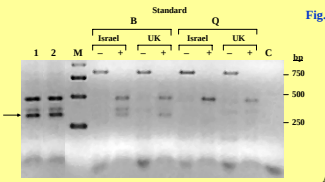

Fig. 3. CAPS analysis based on primers complementary to the sodium channel gene sequences (uncut (-) and digested with *Asu*I (+)).

Lanes 1 & 2, samples obtained from Ma`ayan Zevi (Jul 2003) digested with *Asu*I;
M, 1 kb DNA Ladder;
B, samples from reference B biotype from John Innes Center (UK) and from population Pyri-S (Israel);
Q, samples from reference Q biotype from John Innes Center (UK) and from population Pyri-R Israel);
C, a control without DNA;

Arrow marks the position of B specific band.

CAPS on a basis of cytochrome oxidase I (mtCOI) gene sequence

Using primers C1-J-2195 and L2-N-3014 (2) COI gene sequences (816 bp) from two biotypes were amplified (Fig. 4).

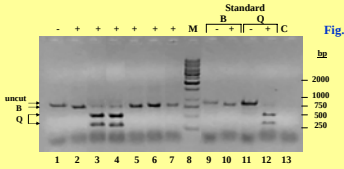

Fig. 4. CAPS analysis based on primers complementary to the mtCOI gene sequences (uncut (-) and digested with *Vsp*I (+)).

Lanes 1 - 7, samples obtained from Ma`ayan Zevi (Jul 2004);
M, 1 kb DNA Ladder;
B, samples from reference B biotype from CNR (Italy);
Q, samples from reference Q biotype from CNR (Italy) ;
C, a control without DNA;

Arrows mark the positions of uncut PCR products, B and Q specific bands.

We developed and applied CAPS on the basis of mtCOI sequences using *Vsp*I. From the PCR products of mtCOI gene (lanes 1, 9 and 11) from whiteflies belonging to biotype B only short fragment (~100 bp) was cut out (lanes 2, 5-7, 10), while PCR products of biotype Q were digested to two fragments: about 500 and 300 bp (lanes 3, 4 and 12), respectively (Fig. 4).

Fig. 5. Provisional distribution of some field populations of *B. tabaci* in Israel described in table 1.

Table 1. Various field populations of *B. tabaci* collected in Israel and diagnosed for biotype status using different techniques

Area and collection site	No. in fig. 5	Date of collection	Crop	Biotype	[b]Technique
Ma´agan Mikha´el	I	Aug 2005	Cotton	Q	1,2, 4
[a]Pyri-S (laboratory)	II	1987	Cotton	B	1, 2, 3, 4
Sha´alvim	II	Jul 2003	Sunflower	Q	1, 2, 3, 4
[a]Pyri-R (laboratory)	III	1991	Rose GH	Q	1, 2, 3, 4
Revivim	III	Sep 2005	Batata (organic)	B	1, 2, 4
Hazeva	IV	Jun 2003	Basil GH	Q	4
Havat Yair	IV	Feb 2004	Sage GH (organic)	B	2, 3, 4
Havat Yair	IV	Dec 2004	Eggplant GH	Q	4
Havat Yair	IV	Dec 2004	Cucumber GH (organic)	B	1, 4
Havat Yair	IV	Feb 2005	Eggplant GH	Q	1, 4
Havat Yair	IV	Jan 2006	Tomato GH (organic)	B	1, 2, 4

[a]Pyri-S and Pyri-R strains were used as standards (1). [b]1, RAPD-PCR; 2, SCAR and CAPS on a basis of RAPD-PCR; 3, CAPS for sodium channel gene; 4, CAPS for mtCOI. [c]GH, greenhouse

Table 2. Comparison of different techniques for detection of *B. tabaci* biotypes

Population	Technique			
	RAPD-PCR	SCAR and CAPS on basis RAPD	CAPS for sodium channel gene	CAPS for mtCOI sequence
Pyri-S (laboratory)	232 B ([a]32)	79 B	55 B	131 B
Pyri-R (laboratory)	177 Q (21)	73 Q	78 Q	143 Q
[b]Ma´ayan Zevi (Jul 2003)	20 B	20 B	15 B	20 B
Sha´alvim (Jul 2003)	10 Q	10 Q	10 Q	20 Q
Me´ir Shefe´ya (Jul 2003)	10 B	10 B	10 B	15 B

[a]In parenthesis, undetectable biotypes (RAPD-PCR). [b]DNA from the same individuals was used.

According to the described methods, the B and Q biotypes are present in Israel. Comparisons studies that were done with the described methods obtained similar results (Table 2). Hence, these methods are suitable for rapid high throughput molecular diagnostic for B and Q biotypes of *B. tabaci*.

Analysis of our recent collections of *B. tabaci* samples taken simultaneously from organic and conventional fields may support our assumptions that the Q-type has higher tolerance to various insecticides than the B-type and the latter exhibits higher fitness in untreated fields (4).

REFERENCES

1. Cenis, J., Perez, P. and Fereres, A. (1993) Identification of Aphid (Homoptera: Aphididae) species and clones by random amplified polymorphic DNA. *Ann. Entomol. Soc. Am.* **86**:545-550.
2. Frohlich, D., Torres-Jerez, I., Bedford, I., Markham, P. and Brown, J. (1999) A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Mol. Biol.* **8**:1683-1691.
3. Horowitz, A.R., Denholm, I., Gorman, K., Cenis, J., Kontsedalov, S. and Ishaaya, I. (2003) Biotype Q of *Bemisia tabaci* identified in Israel. *Phytopar.* **31**:94-98.
4. Horowitz, A.R., Kontsedalov, S., Khasdan, V. and Ishaaya, I. (2005) Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. *Arch. Insect Bioch. Phys.* **58**:216-225.
5. Morin, S., Williamson, M., Goodson, S., Brown, J., Tabashnik, B. and Dennehy, T. (2002) Mutations in the *Bemisia tabaci para* sodium channel gene associated with resistance to a pyrethroid plus organophosphate mixture. *Insect Biochem. Mol. Biol.* **32**:1781-1791.